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Award Number: DAMD17-98-1-8220

TITLE: Characterization of p120ctn, an Adherens Junction Protein
with a Potential Role in Tumorigenesis and Cancer
Metastasis

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REPORT DATE: July 2000

TYPE OF REPORT: Annual Summary

20010504 131

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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**Form Approved
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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE July 2000	3. REPORT TYPE AND DATES COVERED Annual Summary (1 Jul 99 - 30 Jun 00)	
4. TITLE AND SUBTITLE Characterization of p120ctn, an Adherens Junction Protein with a Potential Role in Tumorigenesis and Cancer Metastasis		5. FUNDING NUMBERS DAMD17-98-1-8220	
6. AUTHOR(S) Gordon Polevoy Mark Peiffer, Ph.D.			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of North Carolina Chapel Hill, North Carolina 27599-1350		8. PERFORMING ORGANIZATION REPORT NUMBER	
E-MAIL: mlckl@med.unc.edu			
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012		10. SPONSORING / MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES			
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited		12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 Words) One of the deadliest and least understood aspects of cancer is metastasis. Before a tumor can metastasize, individual cells must acquire mutations which down-regulate adhesion to neighboring cells. Down-regulating components of the adherens junctions causes increased invasiveness and metastatic potential of tumors. Adherens junctions form around cadherins that interact homotypically to cadherins on neighboring cells. The cytoplasmic domain of cadherins interacts with a set of accessory proteins called catenins, which anchor cadherins to the actin cytoskeleton. p120ctn, which was discovered in vertebrates, seems to be playing a regulatory rather than a structural role in adherens junctions. Before we can understand the role p120ctn is playing in cancer, we must understand its normal cellular function. We have been studying p120ctn in the fruitfly, <i>Drosophila melanogaster</i> . The objective of this research project is to characterize the role of p120ctn by generating flies mutant for the p120 gene and characterizing them phenotypically and biochemically. We have generated 200 mutations in the p120ctn region, and have identified a small chromosomal deletion that removes p120ctn and affects only two complementation groups. We are testing these to determine whether either is the p120ctn gene. We are also characterizing anti-p120ctn antisera.			
14. SUBJECT TERMS Breast Cancer, cell adhesion, p120ctn, adherens junctions, cadherin		15. NUMBER OF PAGES 8	
		16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT Unclassified	18. SECURITY CLASSIFICATION OF THIS PAGE Unclassified	19. SECURITY CLASSIFICATION OF ABSTRACT Unclassified	20. LIMITATION OF ABSTRACT Unlimited

FOREWORD

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July 13, 2000
Date

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(5) Introduction:

One of the deadliest and least understood aspects of cancer is metastasis. Before a tumor can metastasize, individual cells must acquire mutations which down-regulate adhesion to neighboring cells. A number of studies have shown that down-regulating components of the adherens junctions, one of the primary cell-cell adhesion systems, causes increased invasiveness and metastatic potential of tumors.

In adherens junctions, the extracellular domains of cadherins form homotypic interactions with neighboring cells. The cytoplasmic tail of cadherin interacts with a class of proteins termed catenins, which associate with the actin cytoskeleton. Regulation of adhesion is an important process for cellular rearrangements such as during development and axon neurogenesis. p120 catenin is a candidate molecule that may function as a modulator of cell adhesion through its interaction with the membrane proximal region of the cadherin cytoplasmic tail. In vertebrates p120 catenin is phosphorylated by tyrosine kinases in response to a variety of growth factors. Correlated with p120 catenin phosphorylation is a breakdown of adherens junctions, rearrangement of the actin cytoskeleton, and a loss of cell-cell contacts. p120ctn becomes highly tyrosine phosphorylated in metastatic, non-adherent cells. We are studying p120 catenin in *Drosophila* where we can take advantage of the many well-characterized tools in this genetic model system. *Drosophila* is an excellent model because flies have adherens junctions which are highly homologous to vertebrate adherens junctions at the molecular level. In addition, we have identified only a single p120ctn homolog in flies, and, with the completion of the genomic sequence, are reasonably certain that there are no others. The objective of this research project is to characterize the role of p120ctn by generating flies mutant for the p120 gene and characterizing them phenotypically and biochemically.

Specific Aim I. Intracellular localization and interaction of *Drosophila* p120ctn with *Drosophila* E-cadherin and other catenins

Specific Aim II. In vivo structure/function analysis of Dp120ctn

Specific Aim III. Transfer of knowledge gained in *Drosophila* to the study of breast cancer.

(6) Body:

Work on this project is a collaboration between Rob Cavallo, the original awardee, who received his Ph.D. in December 1999, Gordon Polevoy, the current PI, and Steve Myster, a postdoctoral fellow in the Peifer lab.

Specific Aim 1

Dp120 specific antibody

The Dp120 C-terminus was used to generate antibodies in both rabbits and rats. On western blots the antibodies are specific for a doublet that migrates between 90 and 100 kD. Affinity purified antibody has been tested on embryos using immunofluorescence confocal microscopy. Dp120 is ubiquitously expressed and localizes to cellular junctions as well as a fair amount of cytoplasmic staining. Interestingly, in early stage embryos a pair of bright staining structures have also been identified. To ensure Dp120 specificity we are staining embryos homozygous for the Df(2R)M41A8 deficiency and evaluating its staining pattern as compared to a control staining pattern.

Specific Aim 2

Identification of deficiencies that remove the *Dp120* gene

The *Drosophila* p120 gene was previously mapped to the 41C region of the right arm of the second chromosome near the centromere by hybridizing a *Dp120* cDNA probe to an ordered P1 genomic library. To more finely map the *Dp120* gene, deficiency strains and balanced stocks that contain mutations that also map to the 41C region were obtained from the Bloomington stock center. Complementation tests were performed to order the deficiencies in relation to each other and in reference to the genetic markers. In preparation for the *Dp120* genetic screen (see below) we performed three experiments designed to determine which of the deficiencies removed the *Dp120* gene. Initially, *in situ* hybridization of polytene chromosomes heterozygous for the deficiency were probed with the *Dp120* cDNA. In polytene chromosomes the two homologues of each chromosome have replicated multiple times without division and are aligned as synaptic pairs. The absence of genetic material due to the deficiency results in a looping out region of the wild-type chromosome. If the *Dp120* gene is missing in the deficiency strain the probe hybridizes to the looped region in the wild-type chromosome. To confirm these results embryos were collected from the outcrossed deficiency strains and RNA *in situ* analysis was performed using an RNA probe generated from the *Dp120* cDNA. One-fourth of the embryos are homozygous for the deficiency chromosome and in strains that remove the *Dp120* gene we saw no *Dp120* transcripts in late stage embryos. Finally, embryos from outcrossed parents were collected and incubated long enough to allow larvae to hatch. Once again one-fourth of the embryos are homozygous for the deficiency and these embryos die before hatching. DNA was isolated from the dead embryos and analyzed by Southern blotting

using the *Dp120* cDNA as a probe and looking for the presence or absence of *Dp120* genomic restriction fragments. Taken together (Fig. 1), these studies revealed that deficiency strains Df(2R)M41A8, Df(2R)M41A4, Df(2R)Nap13, and Df(2R)Nap14 remove the *Dp120* gene and Df(2R)Nap1, Df(2R)Nap2, Df(2R)Nap5, and Df(2R)Nap 9 do not remove *Dp120*.

Genetic screen to generate and identify mutations in the *Dp120* gene

An F2 recessive lethal screen was performed to identify EMS induced mutations that are lethal over deficiency Df(2R)M41A8 (aka Df740). We screened 6,194 chromosomes and recovered 200 new mutants. To effectively manage this large collection of new strains we have focused on the first 100 mutants and taken advantage of two additional deficiency strains that overlap the deficiency used in the screen (Fig. 2). Df(2R)M41A10 (aka Df 741) removes *Dp120* and extends proximal toward the centromere. Df(2R)Nap1 does not remove *Dp120* and extends distally. Each of the new mutants was tested for complementation against these strains and resulted in four subgroups (Fig. 2). Mutant strains in the first class complement Df(2R)741 and fail to complement Df(2R)Nap1. These cannot be mutations in the *Dp120* gene because the Df(2R)Nap1 deficiency does not remove the *Dp120* gene. Nineteen mutants have been placed in this group. 22 mutants comprise the second group. These strains fail to complement both deficiency strains. Mutants in the third class complement the Df(2R)Nap1 deficiency and fail to complement the Df(2R)741 deficiency. New *Dp120* mutations will be in this class and it has 39 members. A fourth class was complemented by both deficiency strains. Nine mutations met these criteria and may possibly represent mutations elsewhere on the second chromosome that result in lethality in combination with the Df(2R)740 deficiency used in the screen.

Identification of the *Dp120* complementation group

In the interval that defines the third subgroup of mutants there are five known complementation groups that are uncloned (IR3, IR23, IR25, m(2)41A and l(2)41Af). Complementation tests have been performed between some of the previously identified complementation groups and my 39 new mutations in the third class. We have generated alleles of IR3 (5 alleles), IR23 (6 alleles), IR25 (13 alleles), and l(2)41Af (1 allele). The remaining 14 mutants are alleles of m(2)41A, and other unidentified complementation groups. The remaining unassigned 14 new mutants have also been tested for complementation against themselves and fall into four separate complementation groups. We have further ordered the mutations in this region by using a set of very small deficiencies in the region. We have determined that Df(2R)Nipped D, Df(2R)Nipped E, and Df(2R)345 remove the *Dp120* gene while Df(2R)Ae, and Df(2R)Nipped C do not remove *Dp120*. We have carried out complementation tests between these deletions and the NC mutations in the region. As a result, we have two remaining complementation groups which are candidates for being mutations in *Dp120*.

We are using two approaches to identify which complementation group represents mutations in the *Dp120* gene. In the first approach we are sequencing the coding region of the *Dp120* gene from a representative of each complementation group. The *Dp120* gene is comprised of four exons and the PCR products span the entire coding region of the *Dp120* gene except the first exon that is predicted to encode the first seven amino acids of the *Dp120* protein. The splicing donor and acceptor sequences of the second, third and fourth exons of the *Dp120* gene are present in the PCR products. All of the PCR products have been cloned and sequence data is being generated. Individual mutations are being reexamined by sequencing an independent clone to rule out PCR error.

The second approach utilizes a *Dp120* transgene driven by the ubiquitin promoter. The transgene is located on the third chromosome and is homozygous viable. Western blots show that the transgene is robustly expressed as compared to protein samples from non-transgenic flies. Both approaches should allow me to identify the *Dp120* complementation group.

Specific Aim III

We have initiated a collaboration with the group of Keith Burridge, to examine the function of fly p120 in cultured mammalian cells. They have found that over-expression of mammalian p120 has a dramatic effect on cell morphology, and that this appears mediated in part by an interaction of p120 with the proto-oncogene Vav2, which acts as a GEF for Rho family GTPases.

Training accomplishments

Rob Cavallo, the original PI of this grant, defended his thesis in December 1999, and received his Ph.D. His thesis was composed in part of the work described above, and thus was supported in part by this grant. A new graduate student, Gordon Polevoy, took over from Rob on the project, and this transfer was approved by the relevant officials of the USAMRMC. Rob presented the work to date at the Era of Hope Meeting in Atlanta in June 2000.

Appendix 1: Key research accomplishments.

- a) We have isolated 200 lethal and visible mutations in the *Dp120* region.
- b) We have mapped *dp120* to a small Deficiency interval that contains only two known complementation groups.
- c) We have generated stocks carrying a Deficiency removing *Dp120* as well as a *dp120* transgene driven by the ubiquitously expressed *ubiquitin* promotor.
- d) We have begun to characterize an anti-Dp120 antibody.

Appendix 2: Reportable outcomes.

Presentations discussing this work.

"Characterization of the Drosophila homolog of p120ctn, a modulator of adherens junctions."
S.H. Myster, R. Cavallo, M. Peifer. 41st Annual Drosophila Research Conference, Pittsburgh PA, April 2000.

"The cloning and characterization of a *Drosophila* homolog of the adherens junction protein p120CTN.", R. Cavallo, S.H. Myster, M. Peifer. Era of Hope, the DOD Breast Cancer Research Program Meeting, Atlanta GA June, 2000.

Degrees supported in part by this work

Ph. D. in Biology Awarded to Rob Cavallo, December 1999, Title: "New Partners for Armadillo in Signal Transduction and Cell Adhesion".

Appendix 3-- Figures

Figure 1. Chromosomal Deficiencies in the *Dp120* region of chromosome arm 2R. Selected complementation groups in the region are indicated above the chromosome, the heterochromatic regions and bands of region 41 are indicated on the chromosome (the oval indicates the centromere), and three Deficiencies in the region are indicated below the chromosome.

Deficiencies in the *Dp120ctn* region

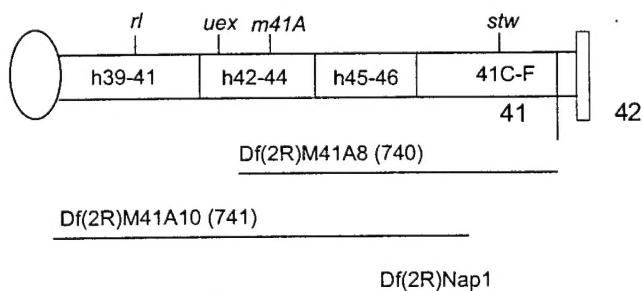


Figure 2. Mapping the alleles generated in the screen to different chromosomal intervals. The screen was done for mutations which fail to complement *Df(2R)740*. These mutations were mapped by complementation with the other two Deficiencies-- The three Deficiencies divide the region up into three regions, based on their overlap with one another. The number of alleles (of the first 100 tested) in each interval is indicated.

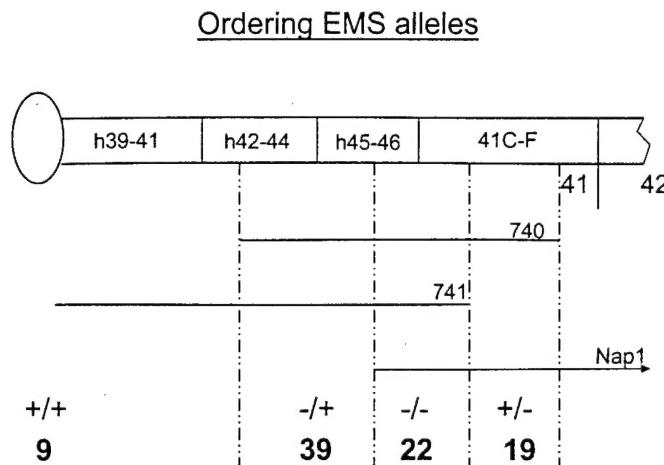


Figure 3 The fine scale genetic map in the *Dp120* region. Above the chromosome are the different complementation groups—if their relative order along the chromosome remains unclear, that is indicated. Below the chromosome are the small Deficiencies used to generate the ordered map. *Dp120* maps into the interval including *l(2R)Af* and *l(2R)NC21*.

